Supporting Information

Juarez *et al.* 10.1073/pnas.0709451105

SI Materials and Methods

Apoptosis Studies. Cells were treated with ATN-224 and cytoplasmic and nuclear fractions prepared and analyzed for the presence of cleaved PARP as described [Juarez JC, *et al.* (2006) *Clin Cancer Res* 12:4974–4982].

Protein Carbonylation. A431 cells were cultured in six-well plates and treated with ATN-224. After 48 h, control cells were treated with 2 mM H_2O_2 and allowed to incubate for 30 min at 37°C. Next, cells were washed with cold PBS, harvested and analyzed by using the OxyBlot protein oxidation detection kit (Chemicon) according to the manufacturer's instructions.

Determination of Redox Status of PTP1B. To determine the redox status of PTP1B, cells that had been treated with ATN-224 for 48 h were washed on ice with degassed PBS. Extracts were prepared with RIPA containing 20 μM Maleimide PEO_2-Biotin (Pierce). Reaction was carried out on ice for 10 min, and 20 mM 2-mercaptoethanol was used to terminate reaction. Samples were immunoprecipitated with an antibody against PTP1B, and Western blot analyses were carried out, probing with avidin-HRP and the same PTP1B antibody.

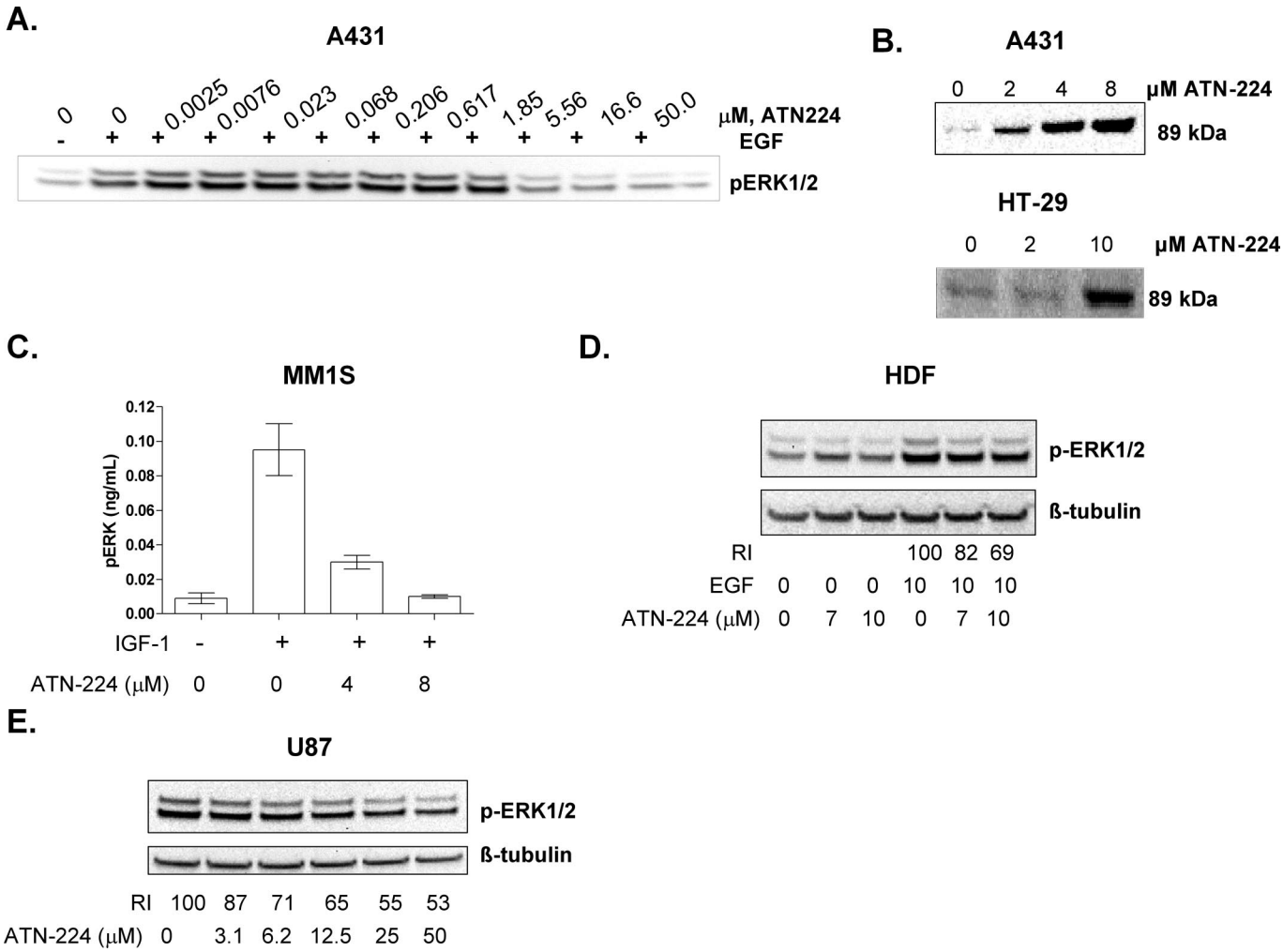

Fig. S1. SOD1 inhibition causes a decrease in phosphorylated ERK1/2 and induces apoptosis. (*A*) A431 cells were treated with increasing concentrations of ATN-224 for 48 h. Then, cells were stimulated with 10 ng/ml of EGF for 10 min and lysed, and Western blot analyses for phosphorylated ERK1/2 were carried out. (*B*) A431 or HT-29 cells in 6-well plates were incubated with ATN-224 for 48 h in full growth media. Then, cells were lysed, nuclear extracts were prepared, and Western blot analyses were carried out, probing for cleaved PARP (89 kDa). (*C*) MM1S multiple myeloma cells were incubated with ATN-224 for 48 h. Then, cells were stimulated with IGF-1 for 10 min and lysed, and ELISAs were carried out. (*D*) Human dermal fibroblasts (HDFs) were incubate with ATN-224 for 48 h, stimulated with 10 ng/ml of EGF for 10 min and lysed, and Western blot analyses for tubulin and phosphorylated ERK1/2 were carried out. The numbers under the bands [relative intensity (RI)] represent the intensity of phosphorylated ERK1/2 bands normalized to tubulin. The average decrease ($n = 3$) in pERK/tubulin in HDFa treated with 10 μM ATN-224 was 25 $\pm$ 11% ($P < 0.01$). (*E*) U87 glioma cells were incubated with the indicated concentrations of ATN-224 for 48 h and lysed, and Western blot analyses for tubulin and phosphorylated ERK1/2 were carried out. The numbers under the bands (RI) represent the intensity of phosphorylated ERK1/2 bands normalized to tubulin.

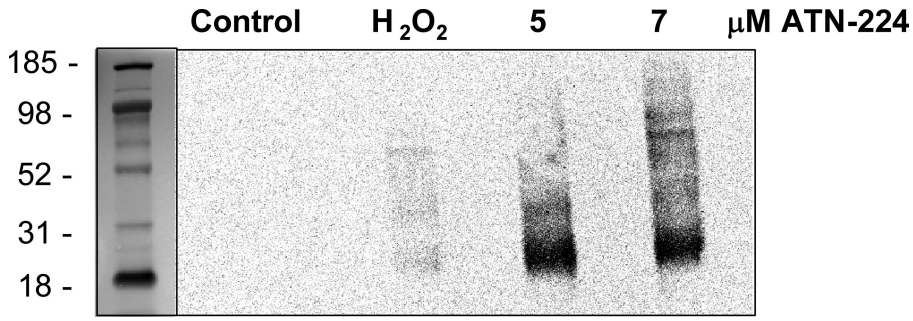

Fig. S2. SOD1 inhibition by ATN-224 increases protein carbonylation. A431 cells were incubated with ATN-224, 2 mM H_2O_2 or buffer (control) for 48 h in full growth media. Then, the presence of protein carbonyls in A431 cells was evaluated by Western blot analysis in controls or cells treated with ATN-224 as described in *Materials and Methods*.

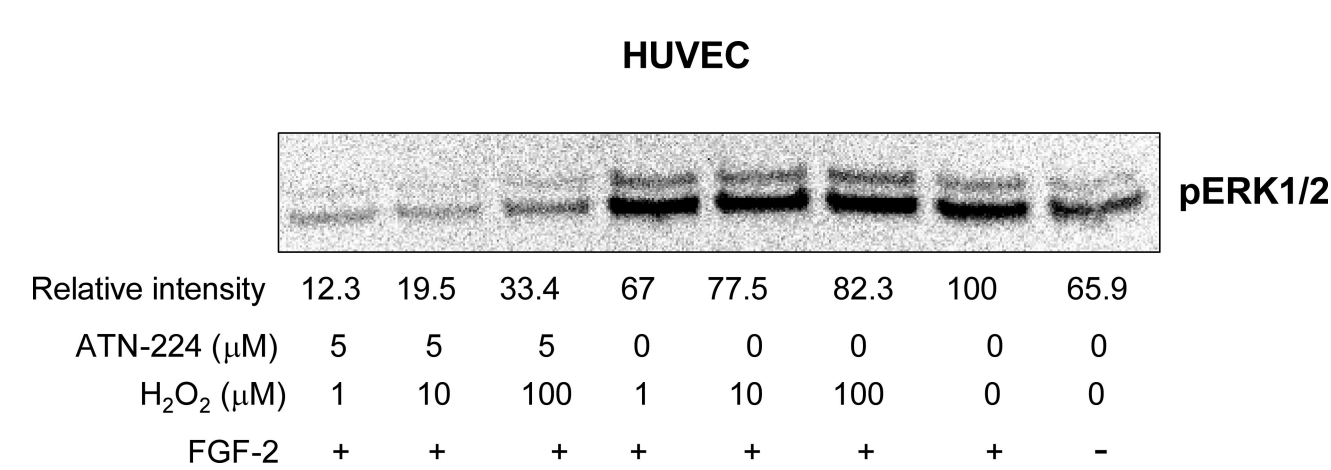

Fig. S3. H_2O_2 partially reverses the inhibition of ERK1/2 phosphorylation upon SOD1 inactivation in HUVEC. HUVEC were incubated with 5 μM ATN-224 for 48 h in full growth media. Then, the cells were treated with 1, 10 or 100 μM H_2O_2 for 0.5 h in cell media containing 0.5% FBS, washed, and stimulated with 10 ng/ml of FGF-2. The numbers under the bands [relative intensity (RI)] represent the intensity of the pERK bands normalized to tubulin.

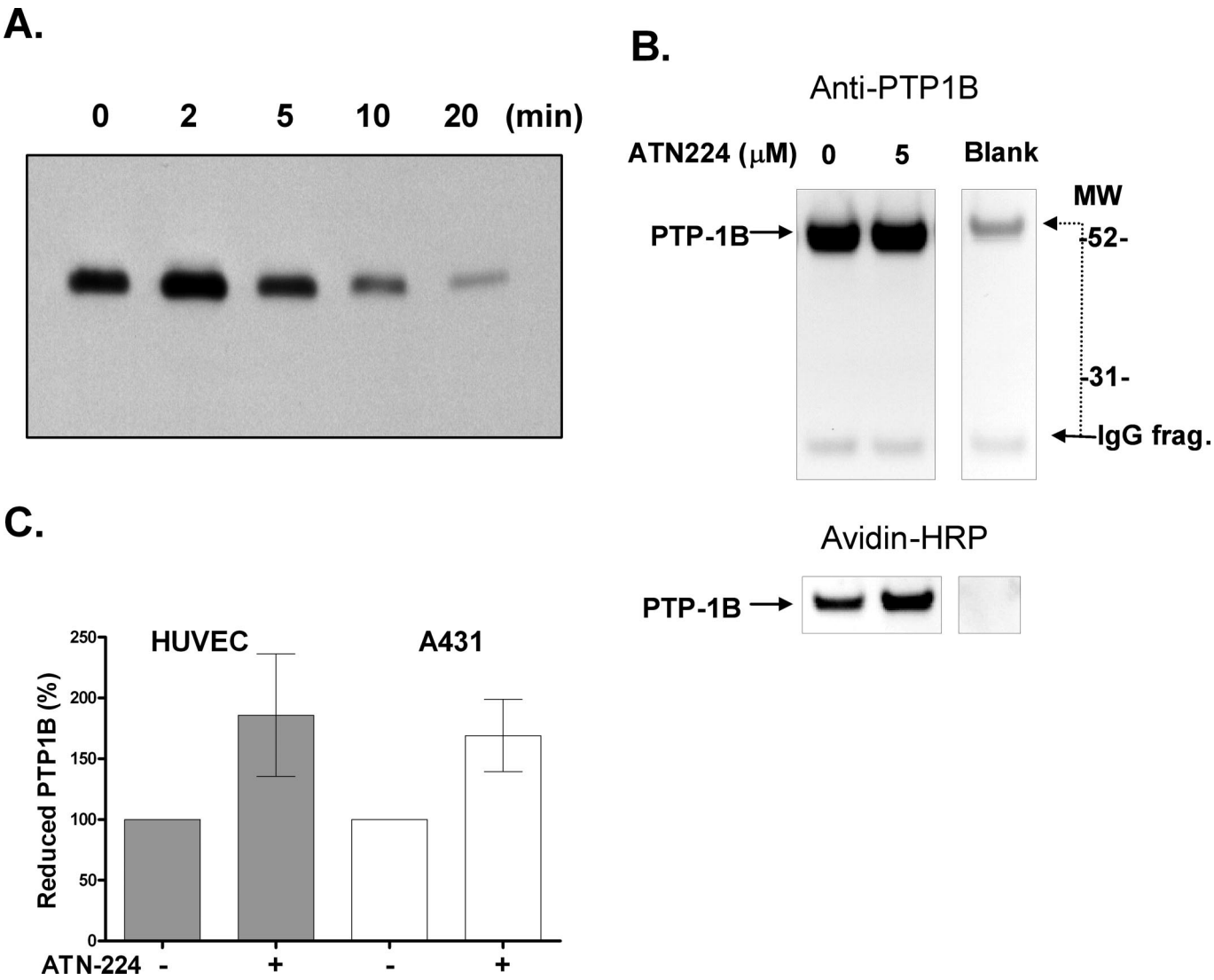

Fig. S4. Inhibition of SOD1 by ATN-224 reduces the levels of oxidized PTP1B. (*A*) A431 cells were incubated with ATN-224 for 48 h in full growth media. Cells were then lysed in the presence of maleimide-biotin, which selectively combines with free Cys residues. The cell lysate was immunoprecipitated (IP) with an anti-PTP1B antibody followed by Western blots (WB) probed for PTP1B or biotin. The blank controls are mock IP's without PTP1B antibody, showing the heavy and light chain of the antibody used for the IP. Quantitation of the PTP1B band showed equal amounts of PTP1B in control and ATN-224 treated A431 cells. (*B*) A431 cells and HUVEC were treated with 5 μM ATN-224 as in *A*, and the bands corresponding to reduced (biotinylated) PTP1B quantitated. The results are shown as the percentage of reduced biotin-containing PTP1B (mean $\pm$ SD, $n = 3$) in the presence and absence of ATN-224.

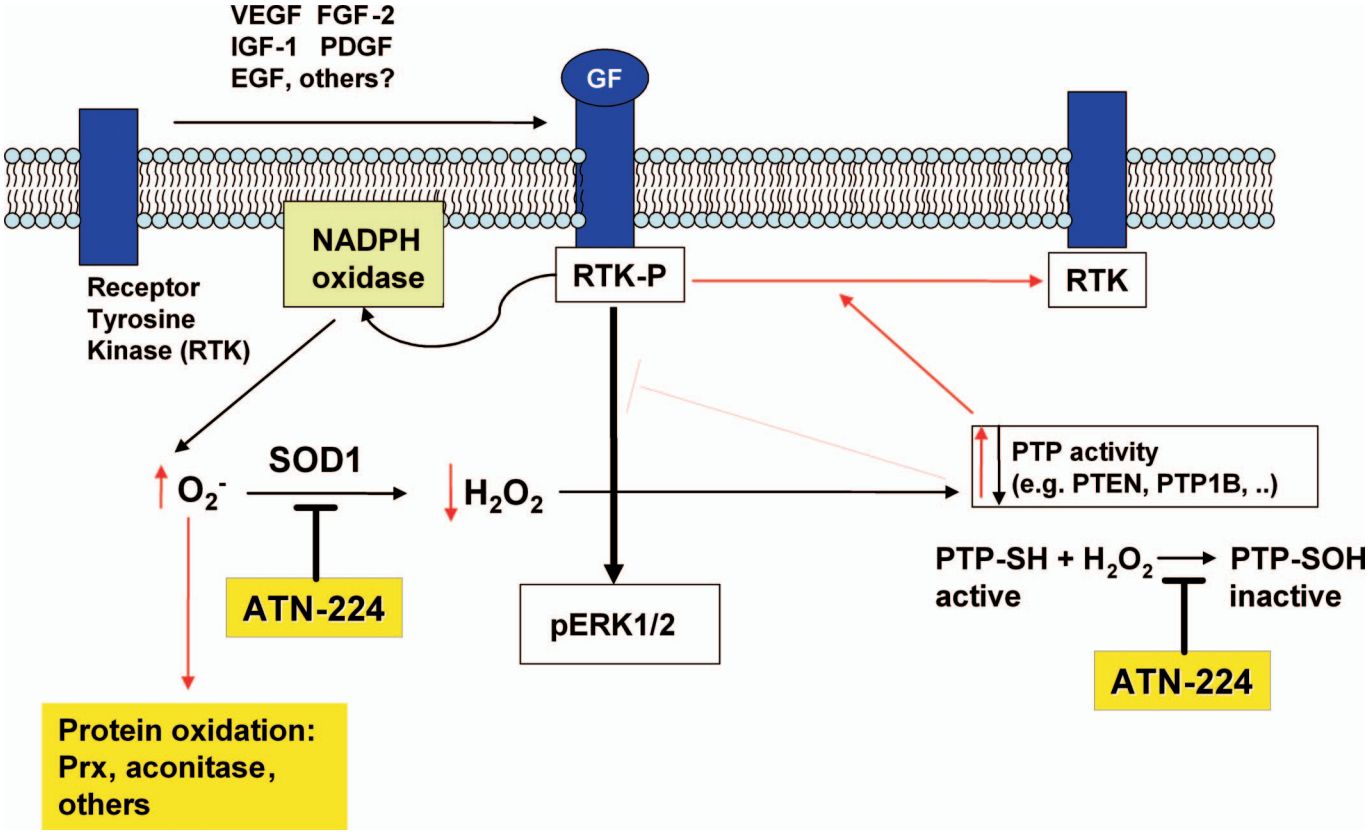

Fig. S5. SOD1 is essential for GF MAKP signaling and inhibition of SOD1 has prooxidant and anti-oxidant effects. Upon GF binding to the receptor, superoxide is generated possibly by the activation of NADPH oxidases. SOD1 catalyzes the dismutation of superoxide into hydrogen peroxide, which in turn inactivates PTPs by reversible oxidation of a Cys in the active site, preventing the de-phosphorylation of substrates activated by kinases. Upon SOD1 inhibition (red dashed lines), H_2O_2 cannot be generated at the levels needed for PTP oxidation and PTPs remain active, counteracting the activity of kinases, which results in the blockage of ERK1/2 phosphorylation. This takes place by the dephosphorylation of the receptor tyrosine kinases by, for example, PTP1B; however, other unidentified PTPs may also be protected and active at different points of the MAKP pathway. The excess superoxide reacts with several proteins inducing protein carbonylation.